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Perilipin Gene Polymorphism and Diabetes Risk in Obese Women

Obez Kadınlarda Perilipin Gen Polimorfizmi ve Diyabet Riski

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Abstract

Purpose: The aim of the study was to investigate the relationship between perilipin gene polymorphisms and diabetes mellitus (DM) type 2 in obese women.

Material and Method: The study population included 21 obese women with type 2 DM, 10 obese women without diabetes. All the coding exons of PLIN gene were amplified by polymerase chain reaction (PCR). Insulin resistance was estimated using the homeostasis model assessment (HOMA).

Results: In obese with type 2 DM, 20 (95.3%) patients were homozygous and 1 (4.7%) was heterozygous for the c.580C>.G (p.Pro194Ala) (rs.6496589) mutations at exon 5 (p= 1.00). As for exon 8, 8 (38.0%) were heterozygous for the c.1113T>C (Pro371Pro) (rs2304796) mutation, and 4 (19.0%) were heterozygous for the c.1113T>C and c.1119C>T (p.Val373Val) (rs2304795) mutations, 6 (28.5%) were homozygous for the c.1113T>C and c.1119C>T mutations and 1 (4.8%) were homozygous for the c.1113T>C mutation (p=0.20). In diabetic obese group, mean levels of HOMA-IR in patients with heterozygous for the c.580C>G mutation at exon 5 and patients with heterozygous for the c.1113T>C and c.1119C>T mutation and patiens with heterozygous for the c.1113T>C mutation at exon 8 were found to be 3.8±1.8 and 4.3±1.7 and 3.5±1.0, respectively.

Discussion: There are no evidences related to perilipin gene polymorphisms such as homozygous for the c.580C>G mutation at exon 5, homozygous for the c.1113T>C and c.1119C>T mutation, and heterozygous for the c.1113T>C at exon 8 associated with diabetes in obese women. *Turk Jem 2012; 16: 58-63*

Key words: Perilipin gene, diabetes mellitus, obesity

Özet

Amaç: Çalışmamızda; obez kadınlarda tip 2 diabetes mellitus ve perilipin gen polimorfizmleri arasındaki ilişkinin araştırılması amaçlandı.

Gereç ve Yöntem: Çalışmaya, 21 tip 2 diyabetli obez ve 10 diyabetik olmayan obez kadın alındı. Perilipin (PLIN) genin tüm ekzonları, polimeraz zincir reaksiyonuyla (PCR) değerlendirildi. İnsülin direnci; homeostasis model assessment (HOMA-IR) yöntemiyle ölçüldü.

Bulgular: Tip 2 diyabetik obezlerde, ekzon 5'de c.580C>.G (p.Pro194Ala) (rs.6496589) mutasyonu için homozigot olan 20 (%95,3) ve heterozigot olan 1 (%4,7) hasta vardı (p= 1,00). Ekzon 8 için, 8 (%38,0) hasta c.1113T>C (Pro371Pro) (rs2304796) mutasyonu için heterozigot, 4 (%19,0) hasta c.1113T>C ve c.1119C>T (p.Val373Val) (rs2304795) mutasyonu için heterozigot, 6 (%28,5) c.1113T>C and c.1119C>T mutasyonu için homozigot ve 1 (%4,8) hasta c.1113T>C mutasyonu için homozigottur (p=0,20). Tip 2 diyabetik obez grupta, ortalama HOMA-IR düzeyleri; ekzon 5'de c.580C>G mutasyonu için homozigot olan hastalarda 3,8±1,8, ekzon 8'de c.1113T>C and c.1119C>T mutasyonu için heterozigot hastalarda 4,3±1,7 ve ekzon 8'de c.1113T>C mutasyonu için heterozigot olan hastalarda 3,5±1,0 olarak bulundu.

Tartışma: Obez kadınlarda; ekzon 5'de c.580C>G mutasyonu içi homozigot, c.1113T>C ve c.1119C>T mutasyonu için homozigot ve ekzon 8'de c.1113T>C mutasyonu için heterozigot gibi perilipin gen polimorfizmleri diyabet ile ilişkili değildir. *Türk Jem 2012; 16: 58-63*

Anahtar kelimeler: Perilipin gen, diabetes mellitus, obezite

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Introduction

Perilipin gene (PLIN) is one established candidate for obesity; it encods an adipocyte-associated protein that influences obesity risk and insulin resistance by regulating adipocyte metabolism, lipolysis, and body fat accumulation (1-5). The relation between adipocyte metabolism and insulin resistance suggests that some of the genetic components involved in insulin resistance may be related to genes primarily expressed in adipocytes (6). Ablation of perilipin resulted in a lean phenotype with a high level of adipocyte lipolysis, enhanced leptin production, and peripheral insulin resistance in animals (7-9). Likewise, significant associations between perilipin genetic variants and body weight in humans have been found in several populations (10-15). On the other hand, associations between insulin resistance and perilipin gene have been investigated in only a few studies (16,17). Qi et al. (16) suggested that central obesity might modify the associations between PLIN variations and diabetes risk in women. In another study, Corella et al. (17) reported that there was no significant association between PLIN 11482G/A and 14995A/T polymorphisms and diabetes or insulin resistance in any ethnic group or sex. However, there are not many studies which reported relationships between perilipin gene and diabetes risk in obese patients. Therefore, the aim of our study was to investigate the relationships between perilipin gene polymorphisms and diabetes risk in obese Turkish women.

Material and Methods

The study population included 21 obese women with type 2 DM (mean age 49.8±9.4 yrs), 10 obese women without type 2 DM (mean age 47.9±9.9 yrs). All the obese women with type 2 DM were previously diagnosed as having diabetes and also, they were on diabetes medication such as metformine (2 gram/day). The control group included obese and normal weight women with normal fasting glucose levels and oral glucose tolerance test data. The study was conducted in accordance with the Helsinki Declaration and it was approved by the local ethics committee. All subjects gave informed consent before they took part in the study.

Anthropometric data (weight, height, and waist and hip circumferences), blood pressure and body mass index (BMI) were recorded for all subjects. A two pole bioelectrical impedance apparatus calibrated for adults (Tanita TBF 300, TANITA Corp. Tokyo, Japan) was used to measure the percent body fat (%BF) and fat mass in all subjects. Biochemical data were obtained using standart methods. Serum concentrations of glucose, triglyceride, total and HDL-Cholesterol were determined by enzymatic procedures. Serum fasting insulin was measured by chemilumminance (determined by means of specific chemiluminescence tests (MLT insulin [intra- and interassay coefficient of variation {CV} 3.8 and 2.3%.) Insulin resistance was estimated using the homeostasis model assessment (HOMA) from fasting glucose and insulin concentrations using the following (18).

$$HOMA\text{-}IR = \frac{(\text{fasting plasma insulin } [\mu U/ml]) \times (\text{fasting plasma glu cose } [mmol/l])}{22.5}$$

Molecular Analysis

Molecular analysis was carried out on genomic DNA extracted from EDTA anticoagulated venous blood using QiAamp DNA Blood Mini kit (QIAGEN GmbH, Hilden Germany) according to manufacturer's directions. All coding exons of PLIN gene (NM_002666.4) were amplified by polymerase chain reaction (PCR) using flanking intronic primers (NCBI Reference Sequence: NC_000015.9.). All synthetic oligonucleotide primers were synthesized from Invitrogen (Invitrogen, Paisley, UK) HPLC purification grade. (Primer details can be obtained from the authors upon request). PCR amplification was carried out on Corbett Palm-Cycler gradient thermal cycler (Corbett Life Science, Australia) in a 25 µl reaction mixture in 0.2 ml thin-wall PCR strip tubes (Axygen Scientific,Inc.,CA, USA) containing 1 µl genomic DNA solution, 1.0 U Platinium TAQ with Enhancer Buffer (Invitrogen Ltd. Paisley, UK), 50 µmol/l each of dGTP, dATP, dTTP and dCTP (Promega, Madison, WI, USA), 5 pmol each of forward and reverse primers. The cycling conditions were set as a hot start at 950C for 10 min, followed by 35 amplification cycles at gradient programme. The amplified PCR products were purified using Exo-SAP PCR purification Kit (Amersham Life Scince) before cycle sequencing reactions. Cycle sequencing PCR was performed using BigDye Terminator v.3.1 kit as per manufacturer's instructions. (PE Applied Biosystems, Foster City, CA, USA). Cycle sequencing of PCR products after purification with BigDyeXT Terminator kit (PE Applied Biosystems, Foster City, CA) were performed using the ABI 3130xl Genetic Analyser System. DNA sequencing was performed in both directions, starting with the forward and the reverse primers used in the initial PCR reaction. SeqScape 2.0 sequencing analysis software was used for sequence evaluation

Statistical Analysis

Statistical analysis was performed using the SPSS for Windows (13.0). Numerical variables were expressed as mean ± standard deviation. The groups were compared using the Mann-Whitney U test. The relationship between diabetes risk and c.580C>G (p.P194A) nonsense polymorphism was evaluated using Fisher's Exact test. In addition,, Chi-Square test was used to evaluate the relationship between diabetes risk and c.1113T>C (p.Pro371Pro) sinonimous amino acid polymorphism.

Results

Clinical characteristics of the study groups are shown in Table 1. Demographic caracteristics, such as mean body weight, BMI, systolic blood pressure, waist circumference were not statistically significantly different between the groups. However, mean level of diastolic blood pressure (p=0.01) and hip circumference (p=0.001) were statistically significantly higher in diabetic obese subjects than in obese subjects without type 2 DM.

Genetic variants of perilipin gene in obese women with and without type 2 DM are shown in Table 2. In obese subjects with and without DM, exon1, exon 2, exon 3, exon 4, exon 6, exon 7 and exon 9 were all found to be wild type. In obese with type 2, 20 (95.3%) patients were homozygous and 1 (4.7%) was heterozygous for the c.580C>.G (p.Pro194Ala) (rs.6496589) mutations at exon 5 (p= 1.00). As for exon 8, 8 (38.0%) were heterozygous for the c.1113T>C (Pro371Pro) (rs2304796) mutation, and 4 (19.0%) were heterozygous for the

c.1113T>C and c.1119C>T (p.Val373Val) (rs2304795) mutations, 6 (28.5%) were homozygous for the c.1113T>C and c.1119C>T mutations and 1 (4.8%) were homozygous for the c.1113T>C mutation (p=0.20). In control, 9 (90.0%) were homozygous for the c.580C>G mutation at exon 5. And also, 4 (40.0%) were heterozygous for the c.1113T>C and c.1119C>T mutations, 4 (40.0%) were homozygous for the c.1113T>C and c.1119C>T mutations at exon 8 in control (Table 2).

Mean levels of cholesterol, glucose, insulin, HOMA-IR, fibrinogen, and hsCRP of the study groups are shown in Table 3. Demographic and biochemical parameters are shown according to perilipin gene variants in obese women with DM and in control subjects in Table 4. Obese women with DM had statistically significantly higher mean levels of glucose (p=0.01), fasting insulin level (p=0.01), HOMA-IR (p=0.01), HbA1c (p=0.01), SGPT (p=0.05), GGT (p=0.01), and hsCRP (p=0.01) than those of nondiabetic obese subjects.

Mean levels of HOMA-IR in patients with heterozygous for the c.580C>G mutation at exon 5 and patients with heterozygous for the c.1113T>C and c.1119C>T mutation and patiens with heterozygous for the c.1113T>C mutation at exon 8 were 3.8±1.8 and 4.3±1.7 and 3.5±1.0, respectively.

Table 1. Demographic characteristics of obese with and without type 2 DM were shown

Parameters	Obese women with DM (n=21) Mean±SD Median (min- max)	Obese women without DM (n=10) Mean±SD Median (min- max)	*p*
Age (yrs)	49.8±9.4 49.6 (31-64)	47.9±9.9 46.5 (30-66)	0.45
Body weight (kg)	95.5±9.9 95.7 (77.0-123.0)	108.5±11.2 104 (98–128)	0.10
Body Mass Index (kg/m^2)	38.6±2.8 38.4 (35.0-45.4)	39.1±2.5 39.0 (34.9-43)	0.52
Waist circumference (cm)	105.5±8.7 107.0 (90-130)	102.2±4.3 100.5 (98-112)	0.28
Hip circumference (cm)	123.0±9.5 121.1 (100-135)	107.7±4.2 106.9 (90-125)	0.01*
Waist/hip ratio	0.84±0.01 0.83 (0.79-0.99)	0.95±0.03 0.94 (0.87-1.1)	0.68
% Fat	42.1±5.3 42.4 (32.8-52.9)	41.7±4.1 41.3 (34.0-50.1)	0.93
Systolic Blood Pressure (mmHg)	126.2±19.8 129.0 (99-142)	129.2±11.5 127.5 (110-180)	0.01*
Diastolic Blood Pressure (mmHg)	71.7±10.7 71 (55-100)	61.0±11.7 57.5 (50-85)	0.45
Duration of disease (year)	8.2±1.1 8.1 (1-17)		
Positive family history (%)	55.4		

*Data are expressed as mean±SD

Table 2. Genetic variants of perilipin gene in obese women with and without type 2 DM and women with normal weight were shown

		Obese with type 2 DM (n=21)	Obese without type 2 DM (n=10)	p values
Exon 5	c.580C>G heterozygous (p.Pro194Ala)	1 (4.7%)	1 (10.0%)	1.00
	c.580C>G homozygous (p.Pro194Ala.)	20 (95.3%)	9 (90.0%)	
	No nucleotide substitution	0 (0.0%)	0 (0.0%)	
Exon 8	c.1113T>C heterozygous (Pro371Pro)	8 (38.0%)	2 (20.0%)	0.20
	c.1113T>C (p.Pro371Pro.) and c.1119C>T (p.Val373Val) heterozygous	4 (19.0%)	4 (40.0%)	
	c.1113T>C (p.Pro371Pro.) and c.1119C>T (p.Val373Val) homozygous	6 (28.5%)	4 (40.0%)	
	c.1113T>C Homozygous (p.Pro371Pro)	1 (4.7%)	0 (0.0%)	
	No nucleotide substitution	3 (9.8%)	0 (0.0%)	

Table 3. Blood profiles of obese patients with and without type 2 DM were shown

	Obese women with DM (n=21) Mean±SD Median (min- max)	Obese women without DM (n=10) Mean±SD Median (min- max)	p
Triglyceride (mg/dl)	156.3±27.2 155.7 (100-230)	133.1±19.5 130.1 (95-166)	0.75
Total-Cholesterol (mg/dl)	222.1±35.0 221.9 (105-320)	205.8±23.1 200.1 (160-285)	0.10
LDL-Cholesterol (mg/dl)	149.5±37.8 149.1 (90-200)	134.0±36.1 130.4 (90-170)	0.34
HDL-Cholesterol (mg/dl)	48.1±7.1 47.9 (35-55)	47.5±2.3 45.2 (30-60)	0.07
Fasting glucose (mg/dl)	126.0±19.8 128.0 (90-165)	91.7±7.8 90.0 (85-100)	0.001*
Fasting insulin (µU/ml)	11.4±5.2 11.0 (3-21)	8.2±2.2 8.5 (5-11)	0.05
HOMA-IR	3.60±1.9 3.8 (1.2-6.9)	1.40±0.4 1.35 (1.0-2.1)	0.001*
SGOT (U/l)	26.2±3.3 26 (17- 41)	22.4±6.3 20.1 (15-39)	0.07
SGPT (U/l)	28.1±3.2 28 (13-88)	23.2±4.4 20.6 (16-30)	0.05*
GGT (U/l)	37.9±1.2 37.5 (25-60)	23.8±3.6 22.9 (18-34)	0.01*
Hematocrit (%)	37.9±1.1 36 (32.1-45.1)	39.0±0.7 38.2 (37.8-43.0)	0.55
Leukocyte (mm^3)	7160.9±1044.3 6500.9 (5300-1070)	7125.0±960.1 7000.1 (7500-9500)	0.73
Platelet (x103/mm^3)	327.0±15.8 327.3 (284-413)	323.1±56.1 323.0 (290-410)	0.15
Urea (mg/dl)	27.5±2.1 27.1 (18-30)	26.3±6.0 25.9 (20-33)	0.41
Creatine (mg/dl)	0.89±0.06 0.90 (0.79-1.0)	0.80±0.09 0. 79 (0.65-0.99)	0.06
HbA1c (%)	7.9±1.3 7.8 (6.1-9.0)	5.5±1.1 5.1 (4.5-6.0)	0.01*

* Data are expressed as mean±SD
Abbreviations: HOMA-IR= Homeostasis model assessment-insulin resistance; LDL= Low-density lipoprotein; HDL= High-density lipoprotein

Discussion

Type 2 diabetes mellitus (DM) is a complex disorder with inherited and environmental factors influencing its occurence. Recently, substantial progress has been made in dissecting a genetic susceptibility to type 2 DM. Genome-wide association studies revealed more than ten genes associated with this disease (19- 21). However, a large part of moleculer background of type2 DM has not yet been described (21). One of the intresting candidates shown in a few studies was the perilipin gene (9). In the present study, we found that type 2 diabetes risk in obese women did not increase with previously reported polymorphisms such as homozygous for the c.580C>G mutation at exon 5, homozygous for the c.1113T>C and c.1119C>T mutation, and heterozygous for the c.1113T>C at exon 8.

The molecular mechanism(s) by which perilipins affect glucose and lipid metabolism in vivo remain unclear. Perilipins increase triacylglycerol storage in adipocytes by forming a physical barrier that reduces the access to soluble lipases on stored lipids (22-26), thus inhibiting triacylglycerol hydrolysis in the basal state (7,22,27) and affect glucose metabolism (9). In a previous report, Qi et al. (11) have showed that there are significant associations between 11482G>A polymorphism and fasting glucose and triacylglycerol concentrations. In addition,, individuals carrying the A allele for the 11482G>A polymorphism are resistant to weight loss with caloric restriction (15). Likewise, Kawai et al. (28) suggested that genetic variation in PLIN1 is associated with glucose intolerance in non-Hispanic white women. However, Mottagui-Tabar et al. (4) reported that two PLIN variants (rs894160 and rs1052700) were not associated with BMI, plasma glucose or triglyceride in obese women. Similarly, Meirhaeghe et al. (29) found that men carrying the rare AA genotype of the rs894160 SNP (also called11482 G>A) had significantly lower fasting plasma glucose levels than GG subjects but this difference was statistically borderline and clinically marginal. Moreover, this polymorphism was associated with type 2 diabetes neither in men, nor in women. To our findings, it was also observed that the frequencies of polymorphisms such as c.580C>G mutation at exon 5, heterozygous for the c.1113T>C mutation, and heterozygous for the c.1113T>C and mutations and homozygous for the c.1113T>C and c.1119C>T mutations at exon 8 were similar in obese subjects with and without type 2 DM.Thus, we did not find any relationship between perilipin gene polymorphisms and diabetes risk in obese women.

The central obesity status significantly interacted with PLIN polymorphisms with respect to diabetes risk (p values for interaction with rs2289487, rs8179043, and rs894160 were 0.027, 0.009, and 0.02, respectively (16). On the other hand, Bergmann et al. (30) reported that polymorphisms in perilipin gene (PLIN) are not associated with obesity and weight variation in people with high risk of type 2 diabetes. In the present study, mean levels of body weight in obese diabetic women with heterozygous for the c.1113T>C mutation at exon 8 have been found to be higher than those of diabetic obese with other polymorphisms. This polymorphism can be attributed to the risk of weight gain in diabetic obese subjects but not to the risk of developing diabetes. And also, to our findings, there was no relationship between waist circumference and gene polymorphism in obese women with type 2 DM.

Studies (17,31) devoted to the investigation of the relationship between perilipin gen polymorphisms and insulin resistance have examined the interaction between macronutrient intake and polymorphisms at the perilipin locus. Corella et al. (17) did not find any significant associations between PLIN 11482G/A and 14995A/T polimorphisms and insulin resistance-related measures (fasting glucose, 2 h glucose, fasting insulin, 2 h insulin and HOMA-IR). However, they found that there was evidence of an interaction between dietary fat (specifically saturated fat) intake, polymorphisms at the perilipin loci (11482G>A, 14995A>T) and insulin resistance. In the present study it was found that diabetic obese

Table 1. Demographic and biochemical parameters were shown according to perilipin gene variants in obese with type 2 DM

Parameters	Obese women with type 2 DM			
	c.580C>G homozygous (exon 5) (n=20) Mean±SD Median (min-max)	c.1113T>C and c.1119C>T homozygous (exon 8) (n=6) Mean±SD Median (min-max)	c.1113T>C heterozygous (exon 8) (n=8) Mean±SD Median (min-max)	No nucleotide substitution (exon 8) (n=3) Mean±SD Median (min-max)
Body weight (kg)	95.2±10.1 95.8 (77.7-123.0)	95.2±11.8 95.1 (76.1-124.9)	102.0±17.2 103 (100-103.3)	96.9±4.0 98 (90-101)
Body Mass Index (kg/m^2)	38.7±2.9 38.4 (35.0-45.4)	38.6±2.8 38.4 (35-44)	40.4±2.4 40.2 (38.2-43)	40.2±3.1 39.1 (37.2-45.4)
Waist circumference (cm)	102.2±4.3 106 (90-130)	105.9±10.9 107.5 (90-130)	102.0±4.0 101 (100-105)	106.6±6.8 107 (100-116)
Hip circumference (cm)	123.5±8.9 124 (101-130)	122.9±20.1 122.8 (95-139)	120.9±15.8 120.1 (100-139)	121.3±11.2 120.7 (111-129)
Fat (%)	42.5±4.1 42.4 (32.8-52.9)	43.4±5.2 38.5 (36.0-44.7)	38.8±4.2 38.4 (34-41.7)	41.1±6.5 40.3 (34.1-50.2)
Systolic blood pressure (mmHg)	126.1±11.8 129.5 (99-142)	125.4±9.8 125 (105-140)	128.8.±7.6 128.7 (110-150)	130.4±13.1 132 (109-142)
Diastolic blood pressure (mmHg)	72.1±10.9 71.5 (55-100)	71.0±13.4 72.0 (55-91)	58.3±7.6 57.8 (50-75)	56.7±6.3 57.1 (45-70)
Fasting glucose (mg/dl)	126.7±20.0 130.0 (90-165)	126.9±17.0 130 (99-154)	127.2±17.0 127.8 (100-135)	115.8±22.2 111.8 (90-143)
Postprandial glucose (mg/dl)	160.2±18.0 182.5 (120-190)	166.5±13.8 166 (145-190)	141.5±11.0 141.7 (125-190)	160.2±18.1 160 (135-185)
Fasting insulin (µU/L)	11.8±5.2 11.0 (3-21)	12.6±5.1 11.5 (3-21)	8.3±3.0 8.5 (5-11)	8.4±1.6 8 (3-9)
HOMA-IR	3.8±1.8 3.8 (1.2-6.9)	4.3±1.7 4.0 (1.2-6.9)	3.5±1.0 3.4 (2.1-4.0)	2.2±1.0 2.1 (1.9-3)
Total-Cholesterol (mg/dl)	220.9±33.6 221.0 (185-300)	205.1±11.1 205.7 (190-290)	200.1±21.7 201.1 (180-270)	210.7±21.5 217.8 (170.1-245)
LDL-Cholesterol (mg/dl)	149.4±20.1 149.8 (90-190)	128.7±14.9 127.8 (95-200)	125.8±30.6 126.0 (95-170)	119.1±23.0 119.8 (80-185)
HDL-Cholesterol (mg/dl)	44.1±7.8 44.0 (35-49)	44.9±12.1 44.1 (39-55)	43.3±7.6 42.9 (35-50)	45.9±9.0 45.0 (40-65)
Triglyceride (mg/dl)	149.2±24.5 149.8 (98-200)	148.5±11.0 148.0 (105-195)	140.0±31.7 140.0 (100-170)	144.5±20.4 144.8 (90-200)

HOMA-IR= Homeostasis model assessment-insulin resistance;
LDL = Low-density lipoprotein; HDL = High-density lipoprotein

women with homozygous for the c.1113T>C and c.1119C> had higher mean levels of HOMA-IR than those of diabetic women with other polymorphisms. This polymorphism might be associated with insulin resistance but not contributed to type 2 diabetes in obese women. Future studies related to relationship between perilipin gene polymorphism and diabetes risk in obese women are needed.

The first limitation of our study may be the small number of subjects involved so that its statistical power may be insufficient to detect small associations. Albeit with a small number of subjects, this was the first study concerning perilipin gene polymorphisms conducted on the Turkish population. The second limitation, we did not investigate the interactions between changing HOMA-IR levels and macronutrient intake with polymorphisms at the perilipin locus. Further studies related to the associations between perilipin locus and insulin resistance and macronutrient intake are needed.

In summary, there were no evidences related to perilipin gene polymorphisms such as homozygous for the c.580C>G mutation at exon 5, homozygous for the c.1113T>C and c.1119C>T mutation, and heterozygous for the c.1113T>C at exon 8 associated with diabetes in obese women.

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